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STEROID BIOSYNTHESIS. THE ABSOLUTE CONFIGURATION OF THE ENZYMATICALLY ACTIVE ENANTIOMER OF 2,3-OXIDOSQUALENE PARTICIPATING IN ITS CYCLIZATION TO LANOSTEROL

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The absolute configuration of the enzymatically active enantiomer of 2,3-oxidosqualene participating in its enzymatic cyclization to lanosterol has been first established experimentally to be 3*S* on the basis of circular dichroism measurements of a $Eu(DPM)_3$ complex of (+)-2,3-dihydroxysqualene derived from the recovered oxidosqualene unchanged.

2,3-Oxidosqualene is known as an important acyclic intermediate in the enzymatic cyclization of squalene to lanosterol[1] and cycloartenol[2] which are the proximal cyclization products in fungi and higher animals and in plants respectively. Although Moss et al.[3] and Cotterrell et al.[4] independently have suggested that 3β-hydroxy triterpenoids may be produced by the cyclization of (3*S*)-2,3-oxidosqualene, this remains to be experimentally proved. We now have established the absolute configuration of natural 2,3-oxidosqualene participating in its enzymatic cyclization to lanosterol on the basis of circular dichroism measurements of a $Eu(DPM)_3$ complex of 2,3-dihydroxysqualene derived from the recovered oxide unchanged.

Racemic 2,3-oxidosqualene[5] ($[\alpha]_D^{25}$ ±0° (*c* 5.21, methanol)[6]; δ($CDCl_3$) 1.23 and 1.27 (s, 1- and 23-CH_3) and 2.69 ppm (t, *J*=7 Hz, 3-CH)) was prepared by alkaline treatment of the bromohydrin of radioactive squalene[7] biosynthesized from (±)-[2-^{14}C]mevalonic acid. The minced hog liver (200 g) in 0.1 M phosphate buffer (200 ml; pH 7.4) was homogenized in a mortar at 0°C. The resulting homogenate was centrifuged for 30 min at 14,000×g and 0°C and the supernatant was used for incubation. The radioactive oxide (300 mg) suspended in the buffer solution (300 ml) containing Tween 80 (600 mg) was anaerobically incubated with the enzyme preparation (200 ml) at 37°C for 2 hr. A product was extracted, after saponification with 1 N methanolic potassium hydroxide, with a 1:1 mixture of ether and *n*-hexane. The radio thin-layer chromatographic analysis, using a silica gel plate (0.25 mm thick) and a 4:1 mixture of ethyl acetate and *n*-hexane as a solvent, revealed the product to be composed of the unchanged oxide with *Rf* value 0.81 and 90% of total counts and lanosterol (II) with *Rf* value 0.56 and 10% of the counts. The recovered oxide (I) unchanged was highly purified by means of tlc and showed the positive optical rotation: $[\alpha]_D^{25}$ +0.73° (*c* 2.73, methanol). Treatment of the (+)-oxide (I) with a 1:10 mixture of 3% perchloric acid and glyme for 2 hr at room temperature afforded (+)-2,3-dihydroxy-

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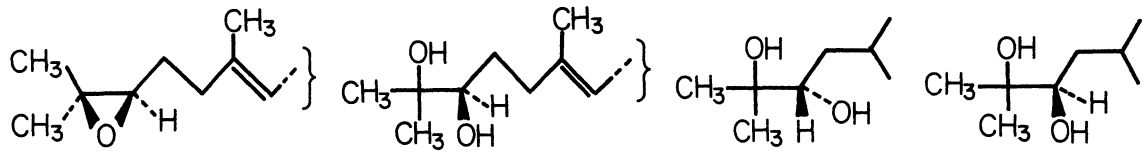
squalene (III): $[\alpha]_D^{25}$ +7.14° (*c* 0.28, methanol); $\delta(CDCl_3)$ 1.14 and 1.18 (s, 1- and 23-CH_3) and 3.30 ppm (dd, *J*=4 and 9 Hz, 3-CH).



(I) (III) (IV) (V)

Since it is well known[8] that such an acidic cleavage of trisubstituted epoxides results in the attack on the tertiary carbon,[8] the configuration at C-3 of the oxide should be retained unambiguously in the dihydroxysqualene (III). Then, the chirality of the dihydroxysqualene (III) was determined by comparing the CD curve of the $Eu(DPM)_3$ complex[9] of III with that of a $Pr(DPM)_3$ complex of an enantiomeric pair, (IV) and (V), of 2,5-dimethylhexane-2,3-diol.[8] The CD curve[6] of the *n*-hexane solution of an equimolar mixture of the dihydroxysqualene and $Eu(DPM)_3$ exhibited the same negative sign in a Cotton effect, $\Delta\varepsilon$(nm) = -0.89 (314), as in the curve of a $Pr(DPM)_3$ complex of the (3*R*)-(+)-dimethylhexanediol (V).[8] The same sign in a Cotton effect, $\Delta\varepsilon$(nm) = -1.21 (316), as above was observed also for the carbon tetrachloride solution. These observations have established the chirality at C-3 of the dihydroxysqualene (III) as *R* and hence the 3*R* configuration for the recovered oxide (I) unchanged. Therefore, the absolute configuration of an enzymatically active, levorotatory enantiomer of 2,3-oxidosqualene participating in its cyclization to lanosterol is 3*S* as shown in VI. The result supports experimentally the biogenesis that 2,3-oxidosqualene (VI) in a chair conformation is cyclized to lanosterol by oxidosqualene-cyclase, as shown in Scheme 1.

(VI) (II)

Scheme 1

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